

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently amended) A method for sequencing a nucleic acid, the method comprising:

providing a substrate which comprises: ~~one or more or more nucleic acid anchor primers;~~
a cavitated fiber optic wafer formed from a fused bundle of a plurality of individual optical fibers, each individual optical fiber having a diameter between 3 and 100 μm , the wafer comprising a top surface and a bottom surface, the top surface comprising at least 10,000 wells, wherein said wells are etched into the top surface of the cavitated fiber optic wafer and wherein the thickness of the wafer between the top surface and the bottom surface is between 0.5 mm and 5.0 mm in thickness; wherein the depth of each well ranges from between one half the diameter of an individual optical fiber and three times the diameter of an individual optical fiber; and wherein a plurality of wells on the top surface of the cavitated wafer have a nucleic acid therein; and a plurality of beads within wells on the top surface of the cavitated wafer, said beads having a pyrophosphate sequencing reagent attached thereto;

delivering additional pyrophosphate sequencing reagents, including sequential delivery of nucleotide triphosphates, from one or more reservoirs to the flow chamber so the beads and nucleic acids in the wells on the top surface of the fiber optic wafer are exposed to the reagents; and

detecting optical signals from each well using a detection means that is in communication with the wells, each optical signal being indicative of reaction of the pyrophosphate sequencing reagents with the nucleic acid in a well, thereby sequencing the nucleic acid.

~~providing a plurality of single stranded circular nucleic acid templates;~~

~~annealing an effective amount of the nucleic acid anchor primer to at least one of the single stranded circular templates to yield a primed anchor primer circular template complex;~~

~~combining the primed anchor primer circular template complex with a polymerase to form an extended anchor primer covalently linked to multiple copies of a nucleic acid complementary to the circular nucleic acid template;~~

~~annealing an effective amount of a sequencing primer to one or more copies of said covalently linked complementary nucleic acid;~~
~~extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer, a sequencing reaction byproduct; and~~
~~identifying the sequencing reaction byproduct, thereby determining the sequence of the nucleic acid.~~

2. (Currently amended) The method of claim 1, wherein said the nucleic acid anchor primer is immobilized on said wells or beads ~~linked to a solid support~~.

3. (Cancelled)

4. (Cancelled)

5. (Cancelled)

6. (Currently amended) The method of claim 1, wherein the ~~circular~~ nucleic acid ~~template~~ is single-stranded DNA.

7. (Cancelled)

8. (Currently amended) The method of claim 1, wherein the ~~circular~~ nucleic acid ~~template~~ is genomic DNA or cDNA.

9. (Currently amended) The method of claim 1, wherein the ~~circular~~ nucleic acid is 10-200 1000 nucleotides in length.
10. (Cancelled)
11. (Cancelled)
12. (Currently amended) The method of claim 1, wherein ~~the sequencing byproduct is~~ pyrophosphate is produced as a sequencing byproduct.
- 13 (Currently amended) The method of claim ~~13~~ 12, wherein the pyrophosphate is detected by contacting the sequencing byproduct with a ~~ATP~~-sulfurylase under conditions ~~sufficient to~~ that allow formation of ATP.
14. (Original) The method of claim 13, wherein the sulfurylase is a thermostable sulfurylase.
15. (Currently amended) The method of claim 12, further comprising adding apyrase to degrade unreacted nucleotide triphosphates.
16. (Currently amended) The method of claim 12, further comprising washing the ~~sequencing product with a wash buffer~~ top surface of the fiber optic wafer with a buffer between each delivery of the nucleotide triphosphates.
17. (Currently amended) The method of claim 16, wherein the ~~wash~~-buffer includes apyrase.
18. (Cancelled)

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19. (Cancelled)

20. (Cancelled)

21. (Cancelled)

22. (Cancelled)

23. (Currently amended) The method of claim 1, wherein the diameter of each individual optical fiber in the cavitated wafer is between 6-50 μm ~~solid support includes at least one optical fiber.~~

24. (Currently amended) The method of claim 1, wherein the nucleic acid sequencing primer is sequenced ~~extended~~ in the presence of a dATP analog.

25. (Original) The method of claim 24, wherein the dATP analog is a thio ATP.

26. (Currently amended) The method of claim 1, wherein the fiber optic surface ~~solid substrate~~ includes two or more nucleic acids ~~anchoring primers~~ separated by approximately 10 μm to approximately 200 μm .

27. (Currently amended) The method of claim 26, wherein the fiber optic surface ~~solid substrate~~ includes two or more nucleic acids ~~anchoring primers~~ separated by approximately 50 μm to approximately 150 μm .

28. (Currently amended) The method of claim 26, wherein the fiber optic surface ~~solid substrate~~ includes two or more nucleic acids ~~anchoring primers~~ separated by approximately 100 μm to approximately 150 μm .

29. (Currently amended) The method of claim 26, wherein the fiber optic surface ~~solid substrate~~ includes two or more nucleic acids ~~anchoring primers~~ separated by ~~approximately 100 μm~~ to approximately 150 μm .

30. (Cancelled)

31. (Cancelled)

32. (Cancelled)

33.-62. (Cancelled)

63. (New) The method of claim 1, wherein said detection means is a CCD camera.

64. (New) The method of claim 1, wherein the substrate has a polished fiber optic surface opposite to the cavitated fiber optic surface.

65. (New) The method of claim 64, wherein the polished surface allows for optical coupling to a second optical fiber.

66. (New) The method of claim 1, wherein the cavitated fiber optic wafer is coated.

67. (New) The method of claim 66, wherein the coating is selected from the group consisting of plastic, gold layers, organosilane reagents, photoreactive linkers, hydrophilic polymer gels and pluronic polymers.

68 (New) The method of claim 1, wherein said pyrophosphate sequencing reagent is luciferase.

69. (New) The method of claim 1, wherein said pyrophosphate sequencing reagent is sulfurylase.

70. (New) The method of claim 1, wherein said substrate further comprises 10^3 or more nucleic acids in said wells.

71. (New) The method of claim 1, wherein said substrate comprises 10^4 or more nucleic acids in said wells.

72. (New) The method of claim 1, wherein said substrate comprises 10^5 or more nucleic acids in said wells.

73. (New) The method of claim 70, wherein the nucleic acids are attached to the wells or beads by a linker.

74. (New) The method of claim 70, wherein the nucleic acids are covalently attached to the wells or beads.

REMARKS

Claims 1-2, 6, 8-9, 12-17, 23-29, and 63-74 are currently pending in the application.

Claim cancellations

Claims 3-5, 7, 10-11, 18-22, and 30-62 have been cancelled without prejudice or disclaimer solely to expedite patent prosecution in accordance with the U.S. Patent Office Business Goals (65 Fed. Reg. 54604 (September 8, 2000)). Applicants reserve the right to present the cancelled claims in a co-pending application.

Claim amendments

Claims 1-2, 6, 8-9, 12-13, 15-17, 23-24, and 26-29 have been amended for clarity, to correct minor typographical errors, and to more fully encompass Applicants' invention. Applicants reserve the right to present any cancelled subject matter in a co-pending application. The claim amendments are supported by the application as originally filed, and do not constitute new matter. Specific supports for the amendments are shown in parentheses, below.

Claim 1 has been amended to recite:

- “a substrate which comprises: a cavitated fiber optic wafer formed from a fused bundle of a plurality of individual optic fibers, each individual optical fiber having a diameter between 3 and 100 μm ” (see, *inter alia*, page 4, lines 8-12 and page 35, lines 18-21 of the originally filed specification);
- “the wafer comprising a top surface and a bottom surface, the top surface comprising at least 10,000 wells, wherein said wells are etched into the top surface of the cavitated fiber optic wafer” (see, *inter alia*, page 4, lines 10-11, page 6, lines 25-26, and page 36, lines 27-29 of the originally filed specification);
- “and wherein the thickness of the wafer between the top surface and the bottom surface is between 0.5 mm and 5.0 mm in thickness; wherein the depth of each well ranges from between one half the diameter of an individual optical fiber and three times the diameter of an individual optical fiber;” (see, *inter alia*, page 36, lines 6-8 and 12-15 of the originally filed specification);
- “and wherein a plurality of wells on the top surface of the cavitated wafer have a nucleic acid therein; and a plurality of beads within wells on the top surface of the cavitated wafer, said beads having a pyrophosphate sequencing reagent attached thereto;” (see, *inter alia*, page 29, lines 8-11 and page 31, lines 8-16 of the originally filed specification);

- “delivering additional pyrophosphate sequencing reagents including sequential delivery of nucleotide triphosphates, from one or more reservoirs to the flow chamber so the beads and nucleic acids in the wells on the top surface of the fiber optic wafer are exposed to the reagents; and” (see, *inter alia*, original Figure 2, as well as page 5, lines 8-10 and page 30, lines 6-7 of the originally filed specification); and
- “detecting optical signals from each well using a detection means that is in communication with the wells, each optical signal being indicative of reaction of the pyrophosphate sequencing reagents with the nucleic acid in a well, thereby sequencing the nucleic acid” (see, *inter alia*, page 4 line 30 to page 5, lines 1-5 and page 32, lines 28-30 of the originally filed specification).

Support for amended claim 1 can also be found in original claims 59-62 (see below).

Claim 2 has been amended to recite “wherein the nucleic acid is immobilized on said wells or beads” (see, *inter alia*, original Figure 4, original claims 59-62 (see below), as well as page 4, lines 9-10 and page 31, lines 8-16 of the originally filed specification).

Claim 6 has been amended to recite “wherein the nucleic acid is DNA” (see, *inter alia*, page 25, lines 6-8 of the originally filed specification).

Claim 8 has been amended to recite “wherein the nucleic acid is genomic DNA or cDNA” (see, *inter alia*, page 9, lines 4-10 and page 17, lines 3-7 of the originally filed specification).

Claim 9 has been amended to recite “wherein the nucleic acid is 10-1000 nucleotides in length” (see, *inter alia*, page 29, lines 13-14 of the originally filed specification).

Claim 12 has been amended to recite “wherein pyrophosphate is produced as a sequencing byproduct” (see, *inter alia*, page 4, line 30 to page 5, lines 1-3 of the originally filed specification).

Claim 13 has been amended to recite “The method of claim 12, wherein the pyrophosphate is detected by contacting the sequencing byproduct with a sulfurylase under conditions that allow formation of ATP” (see, *inter alia*, page 6, lines 7-8 of the originally filed specification).

Claim 15 has been amended to recite “further comprising adding apyrase to degrade unreacted nucleotide triphosphates” (see, *inter alia*, page 6, lines 11-12 of the originally filed specification).

Claim 16 has been amended to recite “further comprising washing the top surface of the fiber optic wafer with a buffer between each delivery of the nucleotide triphosphates” (see, *inter alia*, page 6, lines 10-11 of the originally filed specification).

Claim 17 has been amended to recite “wherein the buffer includes apyrase” (see, *inter alia*, page 6, line 12 of the originally filed specification).

Claim 23 has been amended to recite “ wherein the diameter of each individual optical fiber in the cavitated wafer is between 6-50 μm ” (see, *inter alia*, page 36, lines 1-5 of the originally filed specification).

Claim 24 has been amended to recite “wherein the nucleic acid is sequenced in the presence of a dATP analog” (see, *inter alia*, page 6, lines 4-6 and page 24, lines 11-15 of the originally filed specification).

Claim 26 has been amended to recite “wherein the fiber optic surface includes two or more nucleic acids separated by approximately 10 μm to approximately 200 μm ”; claim 27 has been amended to recite “wherein the fiber optic surface includes two or more nucleic acids separated by approximately 50 μm to approximately 150 μm ”; claim 28 has been amended to recite “wherein the fiber optic surface includes two or more nucleic acids separated by approximately 100 μm to approximately 150 μm ”; and claim 29 has been amended to recite “wherein the fiber optic surface includes two or more nucleic acids separated by approximately 150 μm ” (see, *inter alia*, page 5, lines 14-17 and page 10, line 22 of the originally filed specification).

Entry of the amendments to the claims is respectfully requested.

Newly added claims

Claims 63-74 have been newly added to more fully encompass Applicants' invention. The newly added claims are supported by the application as originally filed and do not constitute new matter. Specific support for the newly added claims is shown in parentheses, below.

New claim 63 recites "wherein said detection means is a CCD camera" (see, *inter alia*, page 32, lines 12-16 of the originally filed specification).

New claim 64 recites "wherein the substrate has a polished fiber optic surface opposite to the cavitated fiber optic surface"; and new claim 65 recites "wherein the polished surface allows for optical coupling to a second optical fiber" (see, *inter alia*, page 36, lines 27-29 of the originally filed specification).

New claim 66 recites "wherein the cavitated fiber optic wafer is coated"; and new claim 67 recites "wherein the coating is selected from the group consisting of plastic, gold layers, organosilane reagents, photoreactive linkers, hydrophilic polymer gels and pluronic polymers" (see, *inter alia*, page 37, lines 3-30 of the originally filed specification).

New claim 68 recites "wherein said pyrophosphate sequencing reagent is luciferase"; new claim 79 recites "wherein said pyrophosphate sequencing reagent is sulfurylase" (see, *inter alia*, page 6, lines 9-10 and page 31, lines 8-16 of the originally filed specification).

New claim 70 recites "wherein said substrate further comprises 10^3 or more nucleic acids in said wells"; new claim 71 recites "wherein said substrate comprises 10^4 or more nucleic acids in said wells"; new claim 72 recites "wherein said substrate comprises 10^5 or more nucleic acids in said wells" (see, *inter alia*, original claims 41-43, 45, and 59 (see below), as well as page 6, lines 25-26, and page 31, lines 8-16 of the originally filed specification).

New claim 73 recites "wherein the nucleic acids are attached to the wells or beads by a linker"; and new claim 74 recites "wherein the nucleic acids are covalently attached to the wells or beads" (see, *inter alia*, page 4, lines 5-7; page 24, lines 10-11; page 31, lines 8-16; and page 37, lines 6-8).

Entry of the newly added claims is respectfully requested.